

# Phosphodiesterase (PDE)4 inhibitors: anti-inflammatory drugs of the future?

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Phosphodiesterase type 4 (PDE4) plays a major role in modulating the activity of virtually all cells involved in the inflammatory process. Inhibitors of this enzyme family display impressive anti-inflammatory and disease-modifying effects in a variety of experimental models. In this review, Mauro Teixeira, Robert Gristwood, Nicola Cooper and Paul Hellewell examine the capacity of PDE4 inhibitors to exert anti-inflammatory actions *in vivo* and discuss the potential of this class of drugs to take their place as novel therapeutic agents for a variety of inflammatory diseases.

Recruitment of leukocytes from the blood compartment into tissues is essential for the host's response to infectious organisms such as bacteria and viruses. If the host's immune and inflammatory responses are properly controlled, the invading microorganism will be destroyed and recuperation of function is virtually complete. However, an initially protective immune response may lead to permanent damage if not controlled, if prolonged or if directed against self. Asthma, arthritis and multiple sclerosis are all examples of chronic immune deregulation accompanied by intense infiltration of tissues with inflammatory cells. In these conditions, chronic inflammation may lead to severe loss of function and to life-threatening situations. Similarly, acute deregulation of the immune system may occur in diseases such as the acute respiratory distress syndrome (ARDS), where an overwhelming and generalized inflammatory response leads to acute incapacitation and frequently to death. For some of these chronic inflammatory conditions (eg. asthma), steroids, sometimes at high doses, are the mainstay of therapy<sup>1</sup>. However, these drugs can have many harmful side-effects when used chronically, including immunosuppression, metabolic disturbances and hypertension. For rheumatoid arthritis, nonsteroidal anti-inflammatory drugs (NSAIDs) offer palliative symptomatic treatment but their known side-effects are of great concern. For other conditions (eg. ARDS), no suitable therapeutic options exist and treatment is largely supportive. Thus, the development of drugs with an effective anti-inflammatory profile, but with fewer side-effects than steroids and the NSAIDs, would be

beneficial as there are few other therapeutic options in a number of diseases where an uncontrolled inflammatory response exists.

A strategy that has received much attention lately, especially within the context of asthma, relates to the level of cAMP in cells that participate in the inflammatory process. The elevation of intracellular cAMP has been associated with inhibition of the function of various types of cells including lymphocytes, monocytes, macrophages, neutrophils, eosinophils, mast cells, basophils, endothelial cells and lung epithelial cells<sup>2,3</sup>. The mechanisms by which cAMP modulates cell function are not completely understood but appear to depend on the activation of protein kinase A and subsequent phosphorylation of hydroxy-amino acid residues or regulatory subunit-dependent transport of cAMP to the cytoplasm and nucleus<sup>4</sup>. The intracellular levels of cAMP are regulated by the rate of cAMP production by receptor-coupled adenylate cyclase and the rate of cAMP degradation by 3',5'-cyclic nucleotide phosphodiesterases (PDEs). Based on genetic, biochemical and pharmacological data, PDE isoenzymes have been classified into seven distinct families<sup>5</sup>. Of these, PDE3, PDE4 and PDE7 appear to be most important for the regulation of cAMP in different cell types. Interestingly, inhibitors of PDE4 have been shown to suppress, with a range of efficacies, the *in vitro* functional responses of most cells involved in the inflammatory process<sup>2,3</sup> (Table 1). Whereas in neutrophils, eosinophils, mast cells and basophils PDE4 isoenzymes play a more dominant role, in monocytes/macrophages and lymphocytes PDE3 isoenzymes are also involved in the regulation of cAMP levels and PDE3 inhibitors appear to synergize with inhibitors of PDE4 (Table 1). The contribution of PDE7 awaits the availability of specific inhibitors of this isoform.

## Effects of PDE4 inhibitors on models of inflammatory diseases *in vivo*

Table 2 describes the effects of PDE4 inhibitors in various 'models' of inflammatory diseases in animals. Despite the spectrum of tissues affected and cell types involved, a consistent finding was that PDE4 inhibitors effectively suppressed inflammation and disease activity. Most of the studies investigating the anti-inflammatory effects of PDE4 inhibitors *in vivo* have focused on allergic diseases, particularly in 'models' of asthma (Table 2). The great interest in allergic diseases is not surprising inasmuch as there is extensive evidence to suggest an involvement of eosinophils in these conditions and PDE4 inhibitors are effective inhibitors of eosinophil activation *in vitro*<sup>6</sup>. In addition, in the context of asthma, PDE4 inhibitors may provide the additional benefit of bronchodilatation and synergism with  $\beta_2$ -adrenoceptor agonists<sup>3,7</sup>. Thus, a number of structurally unrelated PDE4 inhibitors have been shown to suppress eosinophil recruitment induced by antigen challenge and a range of stimuli in the lungs, skin and eyes (Table 2). Furthermore,

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these drugs can also reduce the increased levels of eosinophil-derived secretory products (e.g. eosinophil peroxidase) in the lung and the airway hyper-responsiveness observed after antigen challenge or after exposure to irritants, such as ozone. Interestingly, in some studies<sup>8-10</sup> PDE4 inhibitors preferentially suppressed the recruitment of eosinophils, but not neutrophils, which suggests either a greater sensitivity of eosinophils to inhibition by these drugs or preferential inhibition of an eosinophil-specific recruitment pathway.

A number of investigations have evaluated the effects of various PDE4 inhibitors in animal models of septic shock, particularly in models that use systemic injection of high doses of lipopolysaccharide (LPS) (Table 2). The efficacy of PDE4 inhibitors in these models is very impressive and includes inhibition, very often complete, of LPS-induced increases in serum levels of tumour necrosis factor- $\alpha$  (TNF- $\alpha$ ) in liver injury, bowel injury, lung injury, renal failure and mortality. In the context of acute lung injury, PDE4 inhibitors have been shown not only to inhibit the accumulation of neutrophils but also to reduce neutrophil-dependent oedema and the elevated level of neutrophil-derived elastase in lung tissue.

Three studies have evaluated the effects of the prototype PDE4 inhibitor rolipram on animal models of autoimmune encephalomyelitis (multiple sclerosis), a T-lymphocyte- and TNF- $\alpha$ -dependent demyelinating disease of the CNS (Table 2). Although rolipram had little effect on the induction phase of the disease, it markedly suppressed the pathological, clinical and radiological signs of encephalomyelitis when administered before these symptoms appeared<sup>11-13</sup>. Furthermore, in one study, rolipram also significantly reduced the severity of the disease and inflammatory lesions in the brain when administered after the first clinical signs had appeared<sup>11</sup>.

There is now substantial evidence to suggest that inflammation may play an important role in defining the extent of tissue injury following ischaemia and reperfusion<sup>14</sup>. In this context, rolipram inhibited ischaemia-reperfusion injury in the brain and lung, although it failed to modify injury to the heart (Table 2). The inability of rolipram to modulate myocardial reperfusion injury could relate to the observation of delayed protective effects of prostacyclin analogues in the heart. Following prolonged exposure to 7-oxo-prostacyclin, there is a cycloheximide-sensitive enhanced expression of PDE1 and PDE4 isoenzymes in ventricular muscles of the rat<sup>15</sup>. Thus, on the one hand, the enhanced expression of PDE isoforms leads to attenuation of adrenoceptor-mediated responses and to delayed cardiac protection<sup>16</sup>. On the other hand, inhibition of PDE4 by rolipram could enhance the adrenoceptor-mediated responses and counterbalance any protective anti-inflammatory effect of the drug following reperfusion in the heart.

Anti-inflammatory effects of PDE4 inhibitors have been demonstrated in a rodent model of rheumatoid

**Table 1. Cells involved in the inflammatory process whose functions are known to be suppressed *in vitro* by phosphodiesterase (PDE)4 inhibitors**

Cell type	Activity suppressed	Refs
Neutrophils	Respiratory burst, enzyme release, lipid mediator and cytokine production, phagocytosis, chemotaxis, elevation of free intracellular $Ca^{2+}$ , upregulation of CD11/CD18 expression on the cell surface	36, 63-65
Eosinophils	Respiratory burst, enzyme release, chemotaxis, lipid mediator production, homotypic aggregation, elevation of free intracellular $Ca^{2+}$	66-68
Mast cells	Mediator release	69
Basophils	Mediator release	70, 71
Lymphocytes <sup>a</sup>	Proliferation, cytokine production, cytotoxicity	11, 72, 73
Monocytes/macrophages	Respiratory burst, cytokine production, elevation of free intracellular $Ca^{2+}$	32, 74, 75
Mesangial cells <sup>a</sup>	Proliferation, respiratory burst	76, 77
Endothelial cells <sup>a</sup>	Permeability, expression of cell adhesion molecules, neutrophil adhesion	35, 78

<sup>a</sup>Inhibitory effects can be potentiated by concomitant PDE3 inhibition. For a more complete list of studies, see Refs 3, 63.

arthritis. Both rolipram and CP77059 significantly suppressed ankle swelling and radiological evidence of cartilage injury in a rat arthritis model<sup>17</sup>. In a rat model of glomerulonephritis with mesangial cell proliferation, treatment with rolipram and a PDE3 inhibitor suppressed proteinuria and proliferative changes<sup>18</sup>. In addition, the PDE4 inhibitor Ro20-1724 effectively suppressed the loss of dopaminergic neurones in a mouse model of Parkinson's disease<sup>19</sup>, indicating another disease condition in which PDE4 inhibitors, in theory, have potential utility.

#### Mechanisms of the anti-inflammatory action of PDE4 inhibitors *in vivo*

Inhibitors of PDE4 are effective suppressors of cytokine production by different cell types *in vitro*<sup>2,3</sup> and reduce serum TNF- $\alpha$  levels in animal models of septic shock (e.g. Refs 17, 20, 21) (Box 1). More importantly, inhibition of TNF- $\alpha$  release appears to play an important role in the anti-inflammatory effects of PDE4 inhibitors<sup>22,23</sup>. Suppression of the release of chemoattractants, including the  $\alpha$ -chemokine interleukin-8 (IL-8)<sup>24</sup> and the lipid leukotriene (LT)B<sub>4</sub> (Ref. 25), may also be important for the inhibition of leukocyte recruitment by PDE4 inhibitors. Inhibition of chemokine production, particularly those that are leukocyte-specific chemoattractants, could comprise an important component of the anti-inflammatory action of PDE4 inhibitors *in vivo*.

Pettiphar and colleagues recently showed that the inhibition by rolipram of TNF- $\alpha$  release in the peritoneal cavity of thioglycollate-treated mice was dependent on the release of corticosterone<sup>20</sup>. Similarly, the PDE4 inhibitors rolipram, denbufylline and BRL61063 have all been shown to produce increases in adrenocorticotrophic

**Table 2. The effects of phosphodiesterase (PDE) inhibitors in experimental models of inflammation *in vivo***

Condition modelled	Species	Parameters measured	PDE4 inhibitor used	Route of administration	Effects observed	Refs
Allergic diseases – asthma	Monkey	Antigen-induced BAL eosinophils, IL-1, IL-6, IL-8 and AHR	Rolipram	s.c.	Inhibition	24
	Monkey, guinea-pig	Antigen-induced EPO in lung, bronchoconstriction, BAL neutrophils and eosinophils	CP80633	p.o., s.c.	Inhibition	79
	Guinea-pig	PAF-induced airway oedema	Rolipram	Topical	Inhibition	80
	Guinea-pig	Airway oedema	Rolipram	i.v.	Inhibition	81
	Guinea-pig	Antigen-induced BAL eosinophils, oedema, and AHR	Rolipram	Inhaled/i.p.	Inhibition	26
	Guinea-pig	Antigen-induced BAL eosinophils and histology	Rolipram	p.o.	Inhibition	82
	Guinea-pig	Antigen-induced BAL eosinophils, neutrophils and AHR	Rolipram	i.p. (low doses)	Inhibition of neutrophils	83
	Guinea-pig	Ozone-induced AHR	Rolipram, CDP840, RP73401	p.o./i.p.	Inhibition of AHR CDP840 >> rolipram > RP73401	84
	Guinea-pig	Antigen-induced lung eosinophilia and EPO	Rolipram, RP73401	i.p. (7 days)	Eosinophils inhibited only by RP73401	85
	Guinea-pig	Antigen-induced BAL eosinophils	Rolipram, zardaverine <sup>a</sup>	p.o.	Marginal inhibition	86
	Guinea-pig	IL-5- and IL-8-induced BAL eosinophils	Rolipram, Ro20-1724	p.o.	Inhibition	87
	Guinea-pig	Antigen-induced BAL eosinophils	Rolipram, zardaverine <sup>a</sup>	i.p.	Inhibition after chronic administration only	88
	Guinea-pig	Antigen-induced eosinophils and EPO in BAL	Rolipram, Ro20174	p.o.	Inhibition	89
	Guinea-pig, rat	Antigen-induced BAL eosinophils	Rolipram, RP73401	i.t.	Inhibition (higher doses in rat)	90
	Guinea-pig, rat	Antigen-induced lung eosinophilia and IL-5-induced pleural eosinophilia	Rolipram, CDP840, RP73401	p.o./i.p.	Inhibition CDP840 > RP73401 > rolipram	29
	Rat	Antigen-induced BAL neutrophils and eosinophils	Rolipram, ORG20241 <sup>a</sup>	p.o.	Inhibition	91
	Rabbit	Antigen-induced AHR and eosinophilia	CDP840	i.p.	Inhibition of both and of acute bronchospasm	9
	Rabbit	Antigen-induced AHR and eosinophilia	Rolipram	i.p.	Inhibition of both, no change neutrophils	92
Allergic diseases – eye	Guinea-pig	Histamine- and leukotriene-induced eosinophilia	Rolipram, zardaverine <sup>a</sup>	p.o.	Inhibition	93
Allergic diseases – skin	Guinea-pig	Allergen- and mediator-induced eosinophilia	Rolipram	i.p.	Inhibition	8
Rheumatoid arthritis	Rat	Ankle swelling and radiological evidence	Rolipram, CP77059	p.o.	Inhibition (CP77059 > rolipram)	17
Glomerulonephritis	Rat	Proteinuria, histology	Rolipram and PDE3 inhibitor	Minipump infusion	Inhibition of proteinuria, mesangial proliferation and monocyte infiltration	18

Table 2. The effects of phosphodiesterase (PDE4) inhibitors in experimental models of inflammation *in vivo* (contd)

Condition modelled	Species	Parameters measured	PDE4 inhibitor used	Route of administration	Effects observed	Refs
Septic shock	Mouse	Serum TNF- $\alpha$ , lethality	Rolipram, BRL61063	p.o./i.p.	Inhibition	94
	Mouse	Serum TNF- $\alpha$ , liver injury	Rolipram, zardaverine*	p.o.	Inhibition	95
	Rat	Bowel haemorrhage	Rolipram, denbufylline	i.p.	Inhibition	96
	Dog	Mesenteric hypoperfusion	Denbufylline	i.v. infusion	Reversal	96
	Rat	Serum TNF- $\alpha$	Rolipram	i.v.	Inhibition	21
	Mouse	Serum TNF- $\alpha$ , LPS-induced lethality	Rolipram, CP77059	p.o.	Inhibit TNF- $\alpha$ at lower doses	97
	Mouse	Local and systemic TNF- $\alpha$ , levels	Rolipram	p.o.	Inhibition (locally is dependent on adrenal hormones)	20
	Mouse	Serum TNF- $\alpha$ , lethality	Rolipram, CP77059	p.o.	Inhibition (CP77059> rolipram)	17
Acute respiratory distress syndrome	Rat	Renal blood flow, vascular resistance, glomerular filtration rate	Ro20-1724	i.v. infusion	Reversal of LPS-induced effects	98
	Rat	Serum TNF- $\alpha$ , lethality, pulmonary oedema, liver injury, lung neutrophils	Rolipram	p.o.	Inhibition of all except pulmonary oedema	22
	Rat	Lung neutrophils, elastase activity and AHR	Zardaverine	i.p.	Inhibition	99
	Rat	IL-2-induced pulmonary oedema, lung neutrophils, lung TNF- $\alpha$	Rolipram	i.v.	Inhibition	23
	Guinea-pig	Lung oedema and neutrophils after aerosolised LPS	Rolipram, denbufylline	p.o., i.p.	Inhibition of all but lung neutrophilia	10
Multiple sclerosis	Non-human primate	Clinical signs, MRI, histology	Rolipram	s.c.	Amelioration	12
	Rat	Functional impairment, histology	Rolipram	i.p.	Amelioration and delay in progression	11
	Rat	Functional impairment, histology	Rolipram	i.p.	Delay and slight amelioration when given as preventive treatment; no effect after disease onset	13
Ischaemia-reperfusion injury – brain	Gerbil	Histopathology, hyperthermia	Rolipram	i.p.	Diminished neuronal death	100
Ischaemia-reperfusion injury – heart	Dog	Infarct size and MPO levels	Rolipram	Infusion	No effect	64
Ischaemia-reperfusion injury – lung	Rat	Filtration coefficient, histology, oedema	Rolipram	<i>Ex vivo</i>	Inhibition	101
Parkinson's disease	Mouse	Neuronal loss	Ro20-1724, MNS949	s.c.	Inhibition	19

\*PDE3 and PDE4 inhibitor. BAL, bronchoalveolar lavage; AHR, airway hyperresponsiveness; EPO, eosinophil peroxidase; i.p., intraperitoneal; i.t., intratracheal; i.v., intravenous; LPS, lipopolysaccharide; MRI, magnetic resonance imaging; MPO, myeloperoxidase; PAF, platelet-activating factor; p.o., oral; s.c., subcutaneous; TNF- $\alpha$ , tumour necrosis factor- $\alpha$ . Table 2 contains all references in MEDLINE that report an effect of PDE4 inhibitors on a defined inflammatory condition.

**Box 1. Possible mechanisms involved in the anti-inflammatory action of phosphodiesterase (PDE)4 inhibitors *in vivo***

- Inhibition of the release of inflammatory mediators/cytokines
- Inhibition of leukocyte activation (degranulation, respiratory burst)
- Inhibition of leukocyte migration
- Inhibition of the expression/upregulation of cell adhesion molecules
- Induction of cytokines with suppressive activity (e.g. interleukin-10)
- Induction of apoptosis
- Stimulation of endogenous steroids and catecholamine release

hormone (ACTH) and corticosterone secretion in the rat<sup>27,28</sup>. Moreover, part of the inhibitory effects of rolipram on systemic TNF- $\alpha$  release after LPS treatment was dependent on the release of adrenaline<sup>20</sup> and  $\beta$ -adrenoceptor block reversed the inhibition by rolipram of arachidonic acid-induced oedema in mouse ear<sup>25</sup>. It is clear that these actions should be considered when evaluating the anti-inflammatory actions of these and other PDE4 inhibitors in different animal models. More recently, however, it was reported that the effects of the PDE4 inhibitor CDP840 on IL-5-induced pleurisy in the rat were not modified by adrenalectomy or  $\beta$ -adrenoceptor block<sup>29</sup>. Whether such effects of PDE4 inhibitors occur in humans is unknown.

There are protective effects of PDE4 inhibitors in experimental inflammation that are clearly independent of inhibition of the release and action of TNF- $\alpha$  and other mediators<sup>8,17,30</sup>. For example, rolipram has been shown to inhibit TNF- $\alpha$  release at doses considerably lower than those necessary to prevent lethality following challenge with LPS (Ref. 17) and it also prevented the accumulation of eosinophils induced by intradermal injection of preformed, direct-acting mediators<sup>8</sup>. This latter observation is consistent with a direct effect of rolipram on the eosinophil<sup>31</sup>. It is also clear that cAMP-elevating agents such as PDE4 inhibitors induce the production of IL-10 by macrophages exposed to LPS *in vitro*<sup>32</sup>; the released IL-10 contributes to the inhibitory effects of PDE4 inhibitors on TNF- $\alpha$  and IL-6 release<sup>32</sup>. Although there have been no studies evaluating the role of IL-10 in the anti-inflammatory effects of PDE4 inhibitors *in vivo*, enhanced production of IL-10 appears to play a role in the protective effects of methylxanthines in a murine model of septic shock<sup>33</sup>.

In addition to preventing or inducing the release of cytokines, PDE4 inhibitors potently block the activation of leukocytes *in vitro* (Table 1). It is thus possible that inhibition of leukocyte activation may be important for some of the anti-inflammatory effects of PDE4 inhibitors *in vivo*. For example, in a mouse model of acute lung

injury induced by LPS followed by zymosan, rolipram effectively inhibited lung injury when given before or after the LPS (Ref. 30). This protective effect of rolipram was independent of the inhibition of TNF- $\alpha$  release and of neutrophil sequestration in the lung and also occurred when zymosan was injected alone; this suggests that inhibition of neutrophil activation was the likely mechanism of action. With respect to allergic inflammation, suppression of eosinophil activation in addition to inhibition of mast cell degranulation may play an important role in the protective effects of PDE4 inhibitors in animal models of asthma<sup>29</sup>.

Another interesting and potentially important anti-inflammatory effect of PDE4 inhibitors relates to the ability of cAMP-elevating agents to modulate the expression of cell adhesion molecules *in vitro*. For example, combination treatment with the adenylate cyclase stimulator forskolin and the nonspecific PDE inhibitor isobutyl methylxanthine suppressed the induction by cytokines of endothelial E-selectin and vascular cell adhesion molecule-1 (VCAM-1)<sup>34</sup>. Similarly, rolipram significantly suppressed the expression and release of E-selectin in TNF- $\alpha$ -stimulated human umbilical vein endothelial cells<sup>35</sup>. In addition, cAMP-elevating agents also prevent mediator-induced upregulation of  $\beta_2$  integrins on the surface of eosinophils and neutrophils<sup>36,37</sup>. Whether inhibition of the expression and/or upregulation of cell adhesion molecules plays a role in the anti-inflammatory effects of PDE4 inhibitors *in vivo* is unclear, and deserves further investigation. Finally, the accumulation of leukocytes in different tissues is defined not only by their rate of recruitment into tissue but also by their rate of clearance via apoptotic mechanisms<sup>38,39</sup>. Overall, cAMP-elevating agents tend to enhance apoptosis of various leukocytes *in vitro* (e.g. Refs 40–42). Whether PDE4 inhibitors exert similar effects to other cAMP-elevating agents and whether these will be relevant for their anti-inflammatory action *in vivo* is not known. It is interesting to note that cAMP-elevating agents inhibit neutrophil apoptosis<sup>43</sup>. This finding may provide a possible explanation for the observed lack of effect of PDE4 inhibitors on neutrophil accumulation in some experimental situations (see Table 2).

### Clinical prospects

A major concern that has arisen from the use of PDE4 inhibitors in clinical trials is the ability of these drugs to induce nausea and emetic side-effects<sup>44</sup>. The mechanisms involved in the induction of these side-effects are poorly understood but, based on animal studies, the binding of inhibitors to the so-called rolipram high-affinity binding site is thought to be important (for a review on the high-affinity binding site, see Refs 45–47). Recently this has been addressed formally using a series of biarylcarboxylic acids and amides; a reduction in rolipram binding was associated with a corresponding reduction in emetic effects while anti-inflammatory

potency was maintained<sup>48</sup>. These studies suggest that emetic side-effects can be overcome in clinical practice. However, there are other potential side-effects related to PDE4 inhibition, such as immunosuppression and metabolic disturbances (e.g. altered glucose metabolism; see below). Will chronic administration of PDE4 inhibitors be safer than chronic treatment with steroids or other immunosuppressive agents? Treatment with a PDE4 inhibitor significantly inhibited the *ex vivo* tumoricidal, but not the candididal, activity of macrophages and neutrophils<sup>49</sup>. In addition, systemic treatment with the nonspecific PDE inhibitor theophylline significantly reduced pulmonary antibacterial defence in mice<sup>50</sup>. Phosphodiesterase 4 inhibitors have also been shown to possess significant effects on the release and/or action of hormones such as renin and insulin (e.g. Refs 51–53). Whether *in vivo* treatment with PDE4 inhibitors will result in significant metabolic disturbance clearly deserves further investigation. Moreover, it is important to define the comparative efficacy of PDE4 inhibitors and steroids in different models of inflammation (e.g. Refs 9, 54). As reported with steroids, the effectiveness of PDE4 inhibitors as anti-inflammatory agents may parallel their ability to cause immunosuppression and this needs to be tested experimentally. Such information would help to clarify the indications and potential limitations of these drugs for use in clinical trials. Finally, the prototype PDE4 inhibitor rolipram was initially developed clinically for the treatment of depression<sup>44</sup>. Further investigation is needed to determine whether other PDE4 inhibitors will cause significant CNS effects and whether these will limit their usefulness as anti-inflammatory agents.

### Unanswered questions

Recently, it has become apparent that PDE4 is not just one enzyme but comprises a group of enzymes (PDE4 A–D) which are differentially regulated and expressed in different cells (reviewed in Ref. 45). In general, PDE4 inhibitors have little selectivity for PDE4 subtypes although most appear to be less potent against PDE4C compared with other subtypes<sup>45</sup>. In addition, the expression of the PDE4D isoform is increased following short-term cAMP stimulation and inhibitors that display some specificity for the activated enzyme have been developed<sup>55</sup>. Thus, there is a distinct possibility that the development of specific inhibitors of PDE4 subtypes will become available in the near future. When they do, it will be necessary to assess whether these agents are better than, or at least as effective as, 'nonspecific' PDE4 inhibitors and whether they will induce fewer side-effects.

It is now apparent that chronic activation of inflammatory cells with cAMP is associated with modulation of the activity and numbers of PDE4 isoenzymes. It consists of two regulatory processes: one is short term and involves protein phosphorylation; the other is long term and involves increased gene expression (reviewed in

Ref. 56). More importantly, this modulation of PDE4 isoenzymes is accompanied by a decreased ability of cAMP-elevating agents to inhibit inflammatory cell function and is reversed by rolipram, which suggests that the tolerant state is related to the expression or activity of PDE4 (Refs 57, 58). Interestingly,  $\beta_2$ -adrenoceptor agonists are effective inducers of PDE4 isoenzymes and it is possible that prolonged use of  $\beta_2$ -adrenoceptor agonists may lead to upregulation of PDE4 *in vivo* and the development of tolerance to the anti-inflammatory activities of these drugs<sup>56</sup>. Prolonged inhibition of PDE4 may also lead to upregulation of PDE4 *in vivo* although this requires investigation. Increased expression of PDE4 could result in a state of dependence on PDE4 inhibitors in such a way that it would be difficult to stop patients using the drug. In one study, severe asthmatics that made prolonged use of theophylline could not be weaned off the drug<sup>59</sup>. Clearly, further studies are needed to assess the effects of chronic treatment with PDE4 inhibitors and drug withdrawal in animal models and in the clinical setting. Nevertheless, in view of the capacity of PDE4 inhibitors to reverse tolerance *in vitro*, these drugs may restore responses to cAMP-elevating agents *in vivo*. Finally, atopic patients have increased levels of PDE4 activity when compared to normal individuals<sup>60</sup>. Whether the enzyme(s) that are elevated in atopics are activated or subject to differential inhibition by PDE4 inhibitors is unknown (see Ref. 45).

### Concluding remarks

There has been an enormous excitement, in both industry and academia, with the development of selective PDE4 inhibitors. These are efficacious anti-inflammatory agents in animal models with potential widespread use in diverse inflammatory diseases in humans. Obviously, the answer to whether PDE4 inhibitors will fulfil their promise will only become apparent when clinical trials with appropriate agents have been conducted and reported. No selective PDE4 inhibitors are currently marketed. A number have entered Phase I clinical testing, although most have been dropped subsequently, largely due to side-effects. At present there are two selective PDE4 inhibitors, RP73401 and SB207499, in Phase II clinical testing as anti-asthma agents, and one, LAS31025, further advanced in Phase III. Clinical data on these are eagerly awaited. Recently published data on CDP840 indicate some clinical efficacy<sup>61</sup>, although the level of activity was apparently not sufficient to encourage the continued development of this compound for asthma. Topical application of the PDE4 inhibitor CP80633 significantly reduced inflammation in skin lesions of atopic dermatitis patients<sup>62</sup>. Meanwhile, important questions regarding the mechanism of action *in vivo*, safety and continuous efficacy of PDE4 inhibitors when used chronically remain and should be addressed experimentally.